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Polyfluoroalkyl Compounds in Free-Ranging Bottlenose Dolphins (*Tursiops truncatus*) from the Gulf of Mexico and the Atlantic Ocean

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Polyfluoroalkyl compounds (PFAs) have been used for decades in industrial and commercial products and are now detected worldwide. Concentrations of two major PFA groups, carboxylic acids (PFCAs) and sulfonic acids (PFSAs), were assessed in plasma of bottlenose dolphins from the Gulf of Mexico (Sarasota Bay, FL) and the Atlantic Ocean (Delaware Bay, NJ, Charleston, SC, Indian River Lagoon (IRL), FL, and Bermuda). Eight PFAs were detected in the plasma of all dolphins. Perfluorooctane sulfonate (PFOS) was the predominant compound at all locations (range from 49 ng/g wet weight (w.w.) in dolphins from Bermuda to 1171 ng/g w.w. in plasma of animals from Charleston). Sum of PFA concentrations were significantly higher in animals from Charleston compared to IRL, Sarasota Bay, and Bermuda. Concentrations of several PFAs were negatively associated with age in animals from IRL and Charleston. No differences between gender were observed for all compounds at all locations. An increase in PFA concentrations was associated with a decrease of blubber thickness in animals from Sarasota Bay and IRL. Fluorotelomer 8:2 and 10:2 unsaturated carboxylic acids (FTUCAs), known degradation products of fluorotelomer alcohols and suspected precursors to PFCAs, were detected for the first time at low concentrations in plasma of dolphins.

Introduction

Polyfluoroalkyl compounds (PFAs) are fluorinated chemicals that are chemically/thermally stable and are oleophobic and hydrophobic. There are two major groups of PFAs, the fluorotelomer alcohols and the perfluoroalkyl sulfonamide alcohols, which are produced mainly through telomerization and electrochemical fluorination, respectively. Fluorotelomer alcohols, used as intermediates in the synthesis of perfluoropolymers included in stain repellents, paints, and polishes, have recently been detected in the troposphere (*1*, *2*). Fluorotelomer alcohols have been shown to degrade to carboxylic acids (PFCAs) in the atmosphere and metabolically in animals and microorganisms (*3*−*5*). Fluorotelomer alcohols can also biodegrade to fluorotelomer carboxylic acid (FTCA) which are suspected precursors of PFCAs (8:2 FTCA: $C_8F_{17}CH_2COOH$, 8:2 fluorotelomer unsaturated carboxylic acid (FTUCA): $C_8F_{16}CHCOOH$). Perfluoroalkyl sulfonamide alcohols are known to degrade to sulfonic acids (PFSAs) (e.g. perfluorooctane sulfonate (PFOS)) and are used in a variety of products as stain repellents (i.e. carpet, leather, and textile) and paper protectors. PFOS and fluorotelomer sulfonates are also used in fire-fighting foams. Because of their low vapor pressure and high aqueous solubility, PFCAs and PFSAs are not susceptible to long-range atmospheric transport. However, these contaminants have been globally detected in seawater (*6*, *7*), human blood (*6*, *8*) and wildlife (e.g. fish, birds, polar bears, marine mammals) (*6*, *9*−*11*). It is believed that the neutral and volatile precursors (e.g. fluorotelomer and sulfonamide alcohols) could be responsible for the airborne transport of PFCAs and PFSAs (*12*).

Polyfluoroalkyl compounds have been found to adversely affect both pre- and postnatal development (*13*−*15*) as well as the neuroendocrine system in laboratory animals (*16*). Some PFAs have also shown to inhibit gap junctional intercellular communication (*17*) and cause hepatic peroxisome proliferation in rodents (*18*). In addition, PFAs (>7 carbons) have been shown to bioaccumulate in fish (*19*), and some evidence of PFA biomagnification has been observed between trophic levels (*20*, *21*). The global distribution, the persistence and the toxicity of PFCAs and PFSAs in laboratory animals have led international regulatory agencies to investigate and ban the production/import of some PFAs.

To our knowledge, PFCAs and PFSAs have never been assessed in free-ranging marine mammals. Published reports on PFSAs in cetaceans have been conducted on stranded, hunted, or captive animals. PFOS has previously been reported in liver of stranded dolphins from Florida (*10*). In addition, PFSAs and PFCAs have been detected in tissues of dolphins, harbor porpoises, and sperm whales from the North Sea (i.e. PFOS and PFCAs with 9 to 11 carbons) (*22*), in liver of dolphins and long-finned pilot whales from Italian waters (perfluorohexane sulfonate (PFHxS), PFOS, perfluorooctane sulfonamide (PFOSA), and perfluorooctanoic acid (PFOA)) (*23*) as well as beluga and narwhal livers from the Eastern Canadian Arctic (PFOS, PFOSA, and PFOA) (*21*). PFHxS, PFOS, PFOSA, and PFOA have also been detected in the blood of captive dolphins fed with fish from the Mediterranean and

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TABLE 1. Sampling Information on Bottlenose Dolphins Captured in 2003

location	Sarasota Bay, FL	Bermuda	IRL, FL	Charleston, SC	Delaware Bay, NJ
date	June 2003	June 2003	July 2003	August 2003	September 2003
n	13	2	42	47	5
gender	6F, 7M	1F, 1M	14F, 28M[a]	16F, 31M[a]	2F, 3M
age	3−24		3.5−26[b]	3.5−33[b]	

[a] A male was captured twice. [b] Eight animals of unknown age.

FIGURE 1. Capture and release sampling sites of bottlenose dolphins in 2003.

North Seas (*23*). Although PFA concentrations have been measured, nothing is known about the toxicity of these chemicals to marine mammals.

In this study, we assessed the concentrations of a series of perfluoroalkyl compounds in plasma of bottlenose dolphins (*Tursiops truncatus*) from five geographic areas of the Gulf of Mexico and western Atlantic waters. The relationship between PFA levels and geographic location, age, gender, morphometrics, and blubber thickness of dolphins were investigated.

Materials and Methods

Sample Collection. Blood samples were collected from the fluke vein of free-ranging bottlenose dolphins during capture-release sessions that occurred in 2003 (Table 1, Figure 1) (*24*). Thirteen blood samples were collected in June 2003 in Sarasota Bay, located on the west-central coast of Florida, as part of a health assessment and contaminant study from the Sarasota Dolphin Research Program, a collaborative program involving the Chicago Zoological Society and Mote Marine Laboratory (sampling was performed under the National Marine Fisheries Service (NMFS) Scientific Research Permit No. 522-1569 issued to R. Wells). Blood samples (n=42) were also collected from animals inhabiting the Indian River Lagoon (IRL), located on the east coast of Florida as well as waters surrounding Charleston, SC (n=47) in July and August 2003, respectively. Collections of samples for these sites were conducted by the Harbor Branch Oceanographic Institution and the National Ocean Service as part of the bottlenose dolphin health and risk assessment project (NMFS Scientific Research Permit 998-1678 issued to G. Bossart). In addition, two blood samples were collected in Bermuda by Dolphin Quest researchers in July 2003, and five blood samples were collected by National Marine Fisheries Service scientists from animals in the Delaware Bay, NJ (September 2003). Blood samples were centrifuged immediately after collection during the IRL and Charleston captures and within a few hours of collection for the other sites. Plasma was transferred into polypropylene cryovials which were kept frozen at −20 °C until analysis. Age was estimated from prior knowledge of the animals since birth (Sarasota Bay) or by examination of dentine layers in an extracted tooth (*25*). Measurements of blubber thickness at different body locations were conducted by ultrasound. Eleven measurements were available for Charleston and IRL (i.e. post-nuchal fat pad, external/internal measurements for dorsal, mid-lateral, mid-ventral anterior to blowhole, and mid-ventral ventral to blowhole) and two measurements available for Sarasota Bay (blubber biopsy site and abdomen). Blubber thickness measurements were analyzed individually and averaged together for each animal and assessed in relation to PFA plasma levels.

Standards and Chemicals. Standards of PFHxS (99.99%), PFOS (98%), PFOA (96%), perfluorononanoic acid (PFNA, 97%), perfluorodecanoic acid (PFDA, 98%), perfluoroundecanoic acid (PFUA, 95%), perfluorododecanoic acid (PFDoA, 95%), and perfluorotetradecanoic acid (PFTA, 97%) were purchased from Sigma-Aldrich (Oakville, ON, Canada). PFOSA (99.9%) was provided by 3M (St. Paul, MN). The 8:2 and 10:2 fluorotelomer carboxylic acid (8:2 and 10:2 FTCA) and unsaturated carboxylic acid (8:2 and 10:2 FTUCA) standards (all >95%) were synthesized as described by Achilefu et al. (*26*). Shorter-length perfluoroheptanoic acid (PFHpA, 99%) (Sigma-Aldrich), which is not expected to bioaccumulate, was used as the internal standard. 1,2-^{13}C PFOA (98%) (PerkinElmer Life and Analytical Sciences Canada) was used as an internal standard for Bermuda samples as it became commercially available at the end of the experiment. Methyl *tert*-butyl ether (MTBE, 99.8%), methanol (99%), tetrabutylammonium hydrogensulfate (TBAS, 97%), and ammonium acetate (99+ %) were purchased from Sigma-Aldrich and anhydrous sodium carbonate from J. T. Baker (Phillipsburg, NJ).

Sample Extraction. A liquid−liquid extraction including an ion-pairing agent was used to extract the perfluoroalkyl compounds from plasma (*27*). Plasma (1−1.5 g) was placed into a 15 mL plastic centrifuge tube with 100 μL of the internal standard PFHpA or ^{13}C PFOA (respective final concentration of 25 ng and 20 ng), 3 mL of Na_2CO_3 (0.25 M), and 2 mL of ion-pairing agent TBAS (0.5 M adjusted to pH 10). Two method blanks were created with 1−1.5 g of deionized water for each batch of samples analyzed. Field blanks consisting of deionized water were also analyzed for each location in order to monitor for possible contamination during sample collection and transport. Samples were then extracted with 5 mL of MTBE by shaking vigorously for 10 min and then centrifuged at 1800 rpm for 10 min to isolate the organic phase. The supernatant was collected in a different plastic tube, and the extraction was repeated once more. Supernatants were combined after the second centrifugation. Samples were blown to dryness under high-purity nitrogen gas, diluted in 100% methanol to a final volume of 1 mL, and vortexed for 30 s. The extract was filtered through a 0.2 μm nylon filter into a chromatography vial for analysis.

HPLC-MS/MS Analysis. Concentrations of perfluoroalkyl compounds in blood plasma of dolphins were determined by high-performance liquid chromatography with negative electrospray tandem mass spectrometry (HPLC-MS/MS). Samples were injected with an Agilent 1100 autosampler. The water/methanol (0.01 M ammonium acetate) mobile phase was delivered at a flow rate of 250 μL/min by an Agilent 1100 binary pump. Elution gradient started at 20% methanol, increased to 100% methanol at 5 min, and reverted to the initial conditions after 12 min. Chromatography was performed on a Luna 3μ C8 column (50 × 2 mm, Phenomenex, Torrance, CA) kept at a temperature of 30 °C. HPLC-MS/MS

detection was done with a negative ion turboelectrospray API 2000 (Applied biosystems-MDS Sciex). Data acquisition was done by tandem mass spectometry using a multiple reaction monitoring (MRM) method for optimun parent and daughter ions. Ion mass transition for carboxylic acids were as follows: PFHpA (363→319), ^{13}C PFOA (415→370), PFOA (413→369), PFNA (463→419), PFDA (513→469), PFUA (563→519), PFDoA (613→569), perfluorotridecanoic acid (PFTriA) (663→619), PFTA (713→219), perfluoropentadecanoic acid (PFPA) (763→719), 8:2 FTCA (477→393), 10:2 FTCA (577→493), 8:2 FTUCA (457→393), 10:2 FTUCA (557→493), sulfonic acids PFHxS (399→99), PFOS (499→99), and the precursor PFOSA (498→78).

PFA Quantification and Quality Assurance. Data quality assurance and quality control included field and laboratory blanks, matrix spikes, and standard material injection every 10 samples in order to monitor changes in the sensitivity of the instrument. PFA recoveries were tested based on triplicate analysis of plasma spiked at 100 ng (500 ng for PFOS) and extracted with the same analytical procedure. PFA recoveries varied from 71% for 8:2 FTCA to 155% for 8:2 FTUCA (see Supporting Information for details). Mean ^{13}C PFOA recovery in plasma was 93%. Concentrations were not corrected for recoveries. However, when background levels were detected in blanks, sample concentrations were blank-corrected. Also, PFOA concentrations in samples extracted with ^{13}C PFOA were adjusted for the impurity of the standard (6%). Eight point standard curves were prepared, and standards were extracted together with samples and used for quantification. Equations from PFDoA and PFTA standard curves were used for quantification of PFTriA and PFPA, respectively, due to the lack of available standards. A signal-to-noise ratio of 3 was set as the instrument detection limit (IDL). The minimum level of quantitation (ML) (as defined by the EPA guidelines (*28*) as 3*standard deviation of the blanks or lowest standards in the absence of background levels) was calculated at 0.3 ng/g and 0.4 ng/g, respectively, for 8:2 and 10:2 FTUCA. The ML for PFOA, PFNA, PFDoA, PFTA, PFHxS, PFOS, PFOSA, 8:2 and 10:2 FTCA was 0.5 ng/g and 0.8 ng/g for PFDA and PFUA. Detectable responses that were below the IDL were reported as nondetectable (nd). Responses below the calculated ML were reported as < the corresponding ML.

Statistical Analyses. Data normality was tested using the Shapiro-Wilk tests. PFA plasma concentrations were log-transformed to fit the normal distribution. Nondetect values were replaced with a random number below half of the ML for statistical purposes. For log transformed data, linear regression analysis was used to determine relationships between PFA plasma concentrations and age, weight, length, and blubber thickness ($p \leq 0.05$). A Spearman nonparametric test was applied to analyze the effect of blubber thickness on plasma PFAs because log transformations did not normalize all the blubber thickness data. A one-way ANOVA was used to compare means of log PFA concentrations among locations and by gender within locations. A Tukey's test was performed as a post hoc criterion for PFA means to determine differences among locations ($p \leq 0.05$). An ANCOVA model ([logPFA] = age location age·location) was used to analyze for interactions between age and locations. All statistical analyses were conducted with Systat 11 for Windows (Systat software, Inc., Point Richmond, CA, 2004).

Results and Discussion

PFA Geographical Distribution. Five PFCAs (PFOA, PFNA, PFDA, PFUA, and PFDoA) and three PFSAs (PFHxS, PFOS, and PFOSA) were detected in plasma of all dolphins and significant differences among locations were noted (Figure 2, Table 2). PFOS was the predominant compound in plasma at all locations; geometric mean concentrations ranged from 49 ng/g w.w. in dolphins from Bermuda to 1171 ng/g in plasma of dolphins from Charleston (Table 2). PFOS concentrations in plasma of five dolphins from Charleston exceeded the highest standard curve point of 1 μg/g. Smaller extract volumes were reinjected on the HPLC-MS/MS to fit the calibration range of the standard curve. PFOS and ΣPFA concentrations in dolphins sampled in Charleston were significantly greater ($p \leq 0.05$) than animals sampled in IRL, Bermuda, and Sarasota Bay (Table 2).

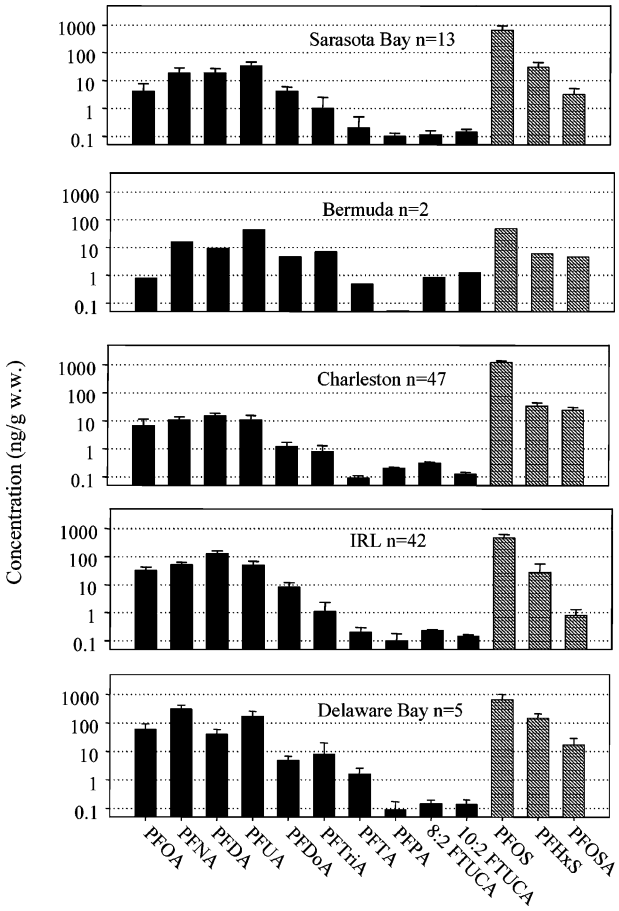

FIGURE 2. Pattern of PFCA (black bars) and PFSA (striped bars) contamination in plasma of bottlenose dolphins from five different sites (geometric means and the upper 95% confidence interval (CI)).

The general pattern of PFCA contamination varied among locations. PFNA was the dominant PFCA in animals from Delaware Bay, PFDA for Charleston, and IRL and PFUA in dolphins from Sarasota Bay and Bermuda. Long-chain PFCAs (>12 carbons) were detected at lower concentrations compared to C_8−C_{11} PFCAs in animals from all geographic sites. These variations in patterns could be due to differences in environmental exposures, diets, or biotransformation activities between dolphins at these different locations.

Significant age and location interactions were associated with PFOA and PFOS concentrations in plasma; thus age adjusted means could not be compared. Geometric means were therefore compared using a one-way ANOVA and Tukey's test. Total PFA concentrations in plasma of dolphins were ranked from highest to lowest as follows: Charleston > New Jersey > Sarasota Bay > Indian River Lagoon >Bermuda (Table 2). Habitat and proximity to sources may be important factors contributing to the exposure of animals to PFAs. Inshore animals inhabiting waters from Charleston, which has the greatest industrial inputs compared to other regions, or the highly residential areas of IRL and Sarasota Bay, are potentially more exposed to contaminants than animals from mid-ocean waters of Bermuda. Dolphins from these coastal locations in the southeastern U.S.A. (i.e.

TABLE 2. Arithmetic Means (ng/g w.w.), Ranges, and Geometric Means ± 95% Confidence Interval of PFA Concentrations Detected in Plasma of Bottlenose Dolphins Captured at Different Geographical Sites

	Sarasota Bay *n*=13			Bermuda *n*=2			IRL *n*=42			Charleston *n*=47			Delaware Bay *n*=5		
PFAs	**arithmetic mean**	**range**	**geometric mean ± CI**	**arithmetic mean**	**range**	**geometric mean ± CI**	**arithmetic mean**	**range**	**geometric mean ± CI**	**arithmetic mean**	**range**	**geometric mean ± CI**	**arithmetic mean**	**range**	**geometric mean ± CI**
PFOA	6.3	0.7–26	4.3 ± 3.5^{c}	0.8	0.6–0.9	0.8^{c-e}	12	1–70	6.9 ± 4.8^{b,c}	44	4.6–163	33 ± 10^{b,e,f}	72	20–115	61 ± 32^{b}
PFNA	25	5.3–74	19 ± 10^{c-e}	17	12–21	16^{d}	13	3–51	11 ± 2.8^{c,d,f}	63	11–214	51 ± 12^{d-f}	326	211–547	305 ± 120^{b,c,e,f}
PFDA	22	5.2–53	18 ± 7.5^{c}	9.6	9.4–9.8	9.6^{c,d}	18	4.3–53	15 ± 3.4^{c,d}	159	41–542	130 ± 31^{b,d,e,f}	45	21–74	40 ± 19^{b,c,e}
PFUA	40	10–94	34 ± 13^{d}	45	43–46	44	15	1.7–64	11 ± 4.7^{c,d}	67	10–320	50 ± 17^{d,e}	192	83–343	170 ± 90^{c,e,f}
PFDoA	5.2	1.3–11	4.3 ± 1.7^{e}	4.6	4.5–4.6	4.6	2	<0.5–6	1.2 ± 0.5^{c,d,f}	13	0.8–62	8.2 ± 3.7^{e}	5.3	3–9	4.9 ± 2^{e}
PFTriA	2.3	nd–8.9	1 ± 1.5^{d}	7.4	5.6–9.2	7.2	1.6	<0.5–7.1	0.8 ± 0.5^{d}	2.7	<0.5–18	1.1 ± 1.2^{d}	13	1.7–37	8 ± 12^{c,e,f}
PFTA	<0.5		<0.5			<0.5	<0.5		<0.5	<0.5		<0.5	1.8	1–4	1.6 ± 1
PFPA	<0.5		<0.5	nd		nd	<0.5		<0.5	<0.5		<0.5	0.07	<0.5–0.22	<0.5
8:2 FTCA	nd		nd	nd		nd	nd		nd	nd		nd	nd		nd
10:2 FTCA	nd		nd	nd		nd	nd		nd	nd		nd	nd		nd
8:2 FTUCA	<0.3		<0.3	0.9	0.5–1.3	0.8	<0.3		<0.3	<0.3		<0.3	nd		nd
10:2 FTUCA	<0.4		<0.4	1.2	1.1–1.4	1.2	nd		nd	nd		nd	nd		nd
PFHxS	40	4.2–111	30 ± 16	5.9	5.5–6.3	5.9^{d}	73	2.2–332	27 ± 29^{d}	46	4.6–165	33 ± 10^{d}	164	48–226	146 ± 60^{b,c,e}
PFOS	780	194–1715	658 ± 262^{c}	49	46–52	49^{c}	642	69–2010	462 ± 164^{c}	1315	472–3073	1171 ± 186^{b,e,f}	751	232–1240	646 ± 348
PFOSA	4.5	nd–14	3.2 ± 2^{c,d}	4.8	3.2–4.5	4.5^{c}	1.5	<0.5–6.5	0.8 ± 0.5^{c,d}	29	7.4–102	24 ± 6^{b,e,f}	20	8.6–44	17 ± 12^{e,f}
ΣPFAs[a]	927 ±573^{c}			139^{c,d}			778 ±66^{c,d}			1738 ±887^{b,e,f}			1588 ±419^{b,e}		

[a] ΣPFAs include PFOA, PFNA, PFDA, PFUA, PFDoA, PFTriA, PFTA, PFPA, PFHxS, PFOS, and PFOSA. [b] Mean is significantly different ($p \leq 0.05$) than Bermuda. [c] Mean is significantly different than Charleston. [d] Mean is significantly different than Delaware Bay. [e] Mean is significantly different than IRL. [f] Mean is significantly different than Sarasota Bay.

FIGURE 3. HPLC-MS/MS responses for 8:2 FTUCA and 10:2 FTUCA in (A) plasma of a Bermuda bottlenose dolphin and (B) 10 ng/uL standard solution.

Charleston, IRL, and Sarasota Bay) are largely known to be resident in these areas year-round (*29*, *30*) and therefore may have continuous exposure to these and other anthropogenic chemicals. Migratory animals, such as dolphins captured in Delaware Bay, were highly exposed to PFAs. These inshore migrants are known to travel along the highly populated U.S. east coast, which is a likely source of exposure. Mean plasma concentrations reported in this study, particularly at Charleston, were generally greater than PFA concentrations reported previously for cetacean tissues and plasma (*10*, *11*, *22*, *23*).

The pattern of PFA contamination at Sarasota Bay, IRL, and Charleston was quite different from previous observations made for polychlorinated biphenyls (PCBs), another group of persistent organohalogens, in surgical and dart blubber biopsies of bottlenose dolphins from the same locations. Based on results published by Hansen et al. (*31*) and Schwacke et al. (*32*), PCB concentrations detected in blubber of male dolphins were higher in Sarasota Bay (mean=76 ng/g of lipid weight (l.w.), *n*=6) followed by Charleston (50 ng/g l.w., *n*=4) and IRL (20 ng/g l.w., *n*=9) (*31*, *32*) which differs from the ΣPFA spatial distribution observed in this study (Charleston>Sarasota Bay>IRL). Compared to PCB concentrations, PFA in plasma showed greater variations between northern and southern sampling locations than PCB concentrations; mean PFA concentrations in animals at Charleston and Delaware Bay were approximately twice as high as compared to Florida animals. This suggests that some PFA sources may be more localized in the northern, more urbanized locations. A pilot study is currently underway to analyze PFAs in water, sediment, zooplankton, and fish samples collected from Charleston Harbor and its tributaries as well as Sarasota Bay.

The 8:2 and 10:2 FTCAs were not detected in this study (<IDL). However, fluorotelomer 8:2 and 10:2 unsaturated carboxylic acids (FTUCA), which are microbial degradation products of fluorotelomer alcohols and have been detected in rainwater (*33*, *34*), are reported here in dolphins. To our knowledge, this is the first report of these PFCA precursors in biota. 8:2 and 10:2 FTUCAs were observed in plasma of dolphins from Bermuda, Sarasota Bay, IRL, and Charleston, with concentrations generally ranging between the IDL and the ML (Table 2). Chromatograms illustrating FTUCA peaks detected in plasma and in a commercial standard demonstrate that these compounds were readily detectable (Figure 3). Detection of fluorotelomer alcohols in air (*1*) as well as FTUCAs and PFCAs in water (*33*) and wildlife (this study) support the hypothesis that telomer alcohols are volatile precursors to FTUCAs that can ultimately biodegrade to PFCAs.

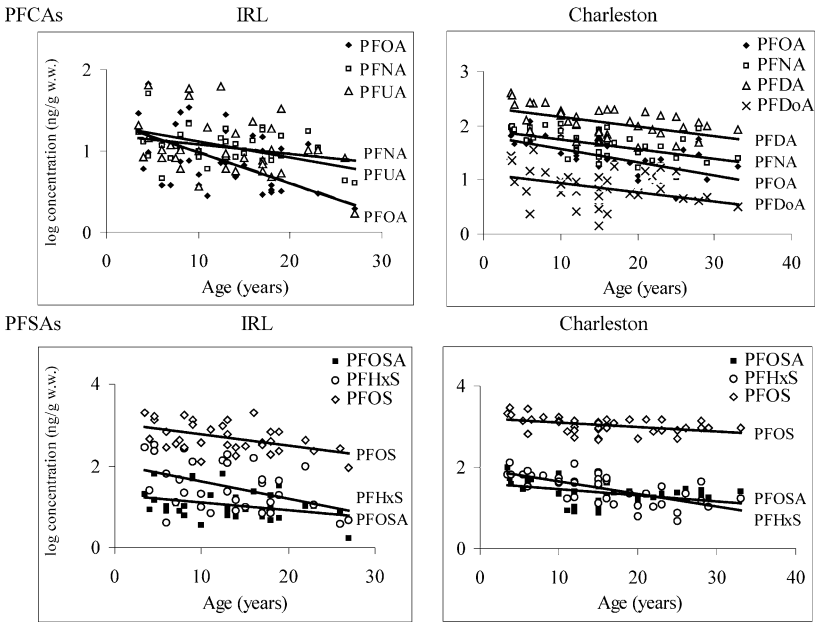

FIGURE 4. Linear regressions between log concentration of PFAs in plasma of dolphins and dolphin's age at IRL and Charleston. PFCAs: Coefficient of determination (R^2) at IRL: PFOA = 0.35, PFNA = 0.11, PFUA = 0.12. Coefficient of determination (R^2) at Charleston: PFDA = 0.35, PFNA = 0.31, PFOA = 0.26, PFDoA = 0.14. For all regressions $p < 0.05$. PFSAs: Coefficient of determination (R^2) at IRL: PFOSA = 0.12, PFHxS = 0.21, PFOS = 0.24. Coefficient of determination (R^2) at Charleston: PFOSA = 0.17, PFHxS = 0.45, PFOS = 0.18. For all regressions $p < 0.05$.

TABLE 3. Correlations between PFAs Detected in Plasma of Bottlenose Dolphins within Locations[a]

	IRL		Charleston		Sarasota Bay		Delaware Bay	
chain length	R^2	p	R^2	p	R^2	p	R^2	p
				PFCAs				
PFOA:PFNA	0.75	<0.005	0.91	<0.005	0.85	<0.005	0.80	0.04
PFNA:PFDA	0.38	<0.005	0.64	<0.005	0.96	<0.005	nr[b]	nr[b]
PFDA:PFUA	0.11	0.03	0.53	<0.005	0.97	<0.005	0.93	0.009
PFUA:PFDoA	nr[b]	nr[b]	0.37	<0.005	0.82	<0.005	nr[b]	nr[b]
PFDoA:PFTriA	0.27	<0.005	0.28	0.001	0.40	<0.005	nr[b]	nr[b]
				PFSAs				
PFHxS:PFOS	0.68	<0.005	0.33	<0.005	0.86	<0.005	nr[b]	nr[b]
PFOSA:PFOS	0.52	<0.005	0.46	<0.005	0.42	0.002	nr[b]	nr[b]

[a] Coefficient of determination (R^2) and probabilities (p) are shown. [b] nr indicates a lack of statistically significant (p>0.05) relationships.

Correlations among PFAs. Statistically significant correlations were found between PFCA and PFSA concentrations within locations at IRL, Charleston, and Sarasota Bay (Table 3). Associations between adjacent PFCAs within sites suggest similar exposure and thus common origins of compounds. In addition, correlations between PFHxS:PFOS and PFOSA:PFOS were observed at all three sites. PFOS is known to be a degradation product of PFOSA (*35*). Results for the interaction PFOSA:PFOS were similar for three locations but absent from Delaware Bay, possibly because of the small sample size. Overall the results for PFSAs suggest a common source for the three chemicals.

Correlation with Biological Parameters. *Gender.* No significant differences in PFA concentrations were observed between genders at any locations, and therefore animals were considered as one group/location for further analyses. A gender difference in PFOA elimination has been observed in rats, where females excreted the compounds more rapidly than males (*36*); however, this has not been noted in any other laboratory species. PFAs have a high affinity for blood and proteinous tissues and are therefore not expected to be transferred in large quantities through milk (*37*). Lack of gender differences has also been reported for PFAs in polar bear liver and PFOS in ringed seal liver (*23*, *38*). In humans, no relationships between PFOS concentrations in plasma and gender have been found in nonoccupationally exposed Canadians (*39*). On the other hand, another human study of American blood donors showed gender differences in mean PFOS concentrations (*40*). Relationships between gender and PFOS concentrations in liver tissue have been reported in harbor porpoises where concentrations in females were higher than in males (*22*). Female harbor porpoises from this latter study are known to feed at a higher trophic level and have a more coastal distribution (*22*) when compared to males. This could explain the higher PFA concentrations.

Age, Weight, and Length. PFOA, PFNA, PFDA, PFDoA, PFHxS, PFOSA, and ΣPFA concentrations in plasma of animals from Charleston and PFOA, PFNA, PFUA, PFHxS, PFOSA, PFOS, and ΣPFA concentrations in dolphins from IRL were negatively correlated with the age of animals (p<0.05) (Figure 4).

Length and weight of animals were also correlated with several PFAs at Charleston and IRL. Concentrations decreased as length or weight of the animals increased (Table 4). No correlation between PFA concentrations and age, length, or weight was observed in animals from Sarasota Bay, Delaware Bay, or Bermuda probably due to the smaller sample sizes. Results from other studies showed no correlation between

TABLE 4. Relationships between Length, Weight, and PFA Concentrations in Dolphin Plasma from IRL and Charleston[a]

	IRL				Charleston			
	weight		length		weight		length	
PFA	R^2	p	R^2	p	R^2	p	R^2	p
PFOA	0.21	0.007	0.23	0.002	0.14	0.02	0.26	0.001
PFNA	0.13	0.04	0.1	0.05	nr	nr	0.18	0.008
PFUA	nr[b]	nr[b]	0.12	0.03	nr[b]	nr[b]	nr[b]	nr[b]
PFDA	nr[b]	nr[b]	nr[b]	nr[b]	nr[b]	nr[b]	0.13	0.03
PFTriA	0.14	0.03	nr[b]	nr[b]	nr[b]	nr[b]	0.10	0.05
PFOSA	nr[b]	nr[b]	nr[b]	nr[b]	0.15	0.02	0.23	0.003
PFHxS	0.27	0.002	0.20	0.004	0.14	0.02	0.24	0.002
PFOS	0.32	0.001	0.22	0.002	nr[b]	nr[b]	nr[b]	nr[b]
ΣPFAs	0.31	0.001	0.22	0.002	nr[b]	nr[b]	0.13	0.03

[a] Coefficient of determination (R^2) and probabilities (p) are shown. [b] nr indicates a lack of statistically significant ($p>0.05$) relationships.

PFOS in liver of gray and ringed seals from the Baltic and Mediterranean Seas and age of animals (*23*). In humans, no association between PFOS in the blood of Japanese donors and age could be found (*6*). As observed at IRL and Charleston, PFA concentrations in dolphin plasma seem to decline with age.

Although no correlations with age could be found for the Sarasota Bay dolphin population, long-term monitoring of this population allowed the comparison of concentrations between known mothers and their calves. Data from the capture of three known mother-calf pairs showed differences in concentrations between mother and calf. ΣPFA levels in a 12-year-old mother was approximately 6 times lower (340 ng/g w.w.) compared to her 3 year-old calf (2000 ng/g). Similarly, ΣPFAs in a lactating 14 year-old mother (225 ng/g) were 10 times lower than in her presumed 5 year-old calf (2100 ng/g) (it is assumed the mother was still nursing this specific calf). An older mother-calf pair (mother 24 year-old, calf 8 year-old) had similar concentrations (1050 and 700 ng/g, respectively). Similarly, PFOS concentrations in the liver of a harbor porpoise foetus (224 ng/g) from Northern Europe showed very high liver concentration compared to its mother (87 ng/g) (*41*). In laboratory studies, PFOS has been shown to cross the placental barrier and compromise the postnatal survival in rodents (*14*). PFOS has also been detected in cord blood samples in pregnant women from Japan (*42*). These studies suggest that PFOS can be transferred in utero to the fetus in humans and rodents. Therefore, this transfer could also be expected in cetaceans. As PFAs are known to bind to proteins, milk also has to be considered. A small number of milk samples were collected from these wild dolphins and will be investigated for PFAs. The possibility of maternal transfer of PFAs to offspring after the first parturition and capacities of aging males to eliminate PFA could explain the similar decrease in PFA concentrations observed for both genders. Changes in the choice of prey and habitat could also explain this decreasing trend in aging males.

Blubber Thickness. A negative relationship was observed in animals from IRL between plasma concentrations of PFOA, PFHxS, and PFOS and thickness of the post-nuchal fat pad (behind the blow-hole) ($p<0.05$) (Figure 5A). At Sarasota Bay concentrations of PFCA, except for PFTA and PFPA, and PFSA concentrations increased as the abdomen blubber thickness decreased ($p\leq0.05$) (Figure 5B). Similar trends were also observed for PFOSA, PFDA, and PFDoA in animals from Charleston for specific blubber thickness measurements (Spearman test, $p\leq0.05$). On the other hand, positive correlations were observed in IRL animals for PFOA, PFUA, and PFOSA as well as dolphins from Charleston for PFOA and PFHxS at specific body locations.

The increasing concentrations in plasma associated with decreasing blubber thickness could suggest, for highly hydrophobic compounds, a mobilization of contaminants from blubber to plasma; however, because of their surfactant-like properties, PFAs are not expected to be associated with lipids or to accumulate in blubber as other organohalogens do. These data suggest that further PFA measurements should be done on lipid rich tissues in cetaceans to determine if there is significant retention. Large seasonal differences in blubber thickness have been observed in Sarasota Bay dolphins (*43*). Relationships between these physiological changes and contaminant burden are presently being evaluated. Van de Vijver et al. (*41*) have observed a lack of correlation between PFOS in harbor porpoise liver and blubber thickness of these by-caught animals. These results are similar to what we observed for a majority of blubber measurements at IRL and Charleston.

In conclusion, a wide spectrum of PFAs was detected in plasma of live-captured and released bottlenose dolphins from five different locations from the western Atlantic Ocean and the Gulf of Mexico. Major differences in PFA concentrations were observed among locations. Dolphins from Charleston had significantly higher plasma levels of PFOS compared to concentrations detected at IRL, Sarasota Bay, and Bermuda. PFA concentrations were not related to gender, but relationships were observed in some instances with age, weight, length, and blubber thickness. Since little is known about the potential health effects of bottlenose dolphin's exposure to PFAs at these concentrations, efforts are being made, as part of these multidisciplinary projects, to assess the possible relationships between PFA plasma concentrations and biomarkers of immune functions, endocrine functions, and general health (i.e. clinical chemistry values, hematology). Finally, potential PFA biomagnification/transfer through the bottlenose dolphin's food web is currently under investigation.

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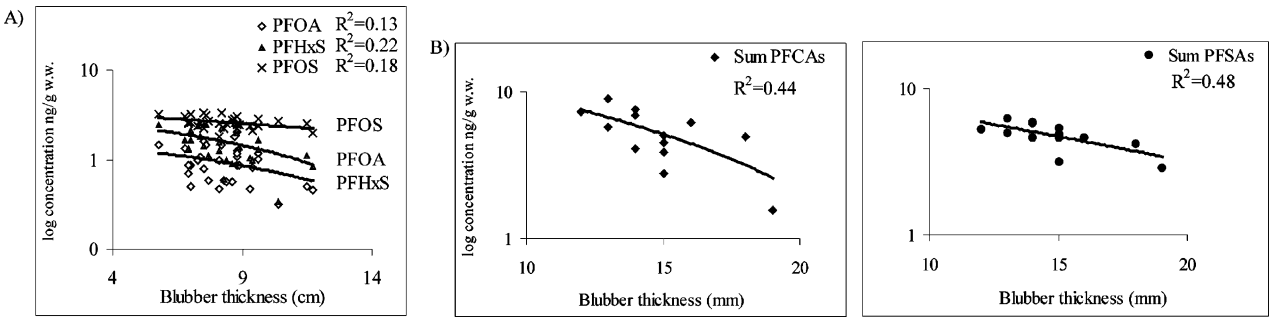

FIGURE 5. Relationships ($p\leq0.05$) between the blubber thickness and log concentration of PFAs detected in dolphin plasma (A) post-nuchal fat pad and PFA plasma concentrations at IRL and (B) abdomen blubber and PFCA/PFSA concentrations at Sarasota Bay.

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Supporting Information Available

Spike concentration (ng), percent recovery, and standard deviation from spike and recovery experiment (n=3) of polyfluoroalkyl compounds in plasma of bottlenose dolphins (Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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